

Pod Borer of Peanut and Potential Entomopathogenic Fungi for its Control in West Sumatera

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Abstract— Pod borer (*Etiella zinckenella*) is a serious pest on peanuts in West Sumatera since the last four years. The larvae lived and bored on pods under soil surfaces and it is often undetected. *E. zinckenella* is very limited reporting as a pest on peanuts especially in Indonesia. We described about pod borer attacking in West Sumatera and as alternative strategic control using entomopathogenic fungi bicontrol agents is safety and more effective and it will be promoted for controlling of larvae in pods under soil. The objective of this research were to determine severity of damage of pod borer with survey and observation methods, and to find out potential entomopathogenic fungi by in both of baiting and dilution method. The severity of attacking of pod borer on groundnut was 14 to 70%. Five group of fungi i.e: *Metharizium sp*, *Aspergillus sp*, *Trichoderma*, *Fusarium* and *Paecilomyces* were isolated from soil of groundnut's rhizosphere in several districts of West Sumatera and they were able to infected of *Tribolium molitor* 8-30%.

Keywords— entomopathogenic fungi, groundnut, rhizosphere, *Etiella zinckenella*

I. INTRODUCTION

Etiella zinckenella (Lepidoptera: Pyralidae) is an important pest. It is known as soybean pod borer. However, in a recent years, this pest has been detecting on peanut (Apriyanto *et al.*, 2008). Actually it never reported in Indonesia (Kalshoven, 1987). The first reporting was from Province of Bengkulu which pod borers have damaged peanuts both at high and low land (Apriyanto *et al.*, 2009). We have found the damage of pod borers on peanut since 2012 in West Sumatera especially in Pasaman Barat (Ganeshi, 2013). This pest due to loss yield significantly if there is no practicing of control at all. Its attacking undetected on peanuts because larvae bore inside pod under soil.

The use of chemicals is tested by farmers proved to be ineffective because the larvae live in pods that can not direct contact with chemicals. In contrast, the use of insecticides can be dangerous because it accumulates in the pod. Alternative controls are considered the most potential to be developed is the use of biological agents especially entomopathogenic fungi. This fungus can be applied directly to the soil around the root zone near the pod. Various types of entomopathogenic fungi can actually last long and multiply so that the potential for infecting larvae survive long in the soil because they are saprophyte.

The initial steps are necessary to take advantage of

entomopathogenic fungi from rhizosphere for pod borer control peanut plants is knowing the diversity of fungi found in peanut cropping land. Several studies entomopathogenic fungi diversity in the rhizosphere of plants has been carried out. Samer research results (2011) indicate that there are four entomopathogenic fungi in the genus rhizosphir chilli crop in several locations in West Sumatra, namely *Fusarium*, *Aspergillus*, *Trichoderma* and *Metarhizium*. Hamdani (2009) further showed that the diversity of entomopathogenic fungi on cacao plantations rhizosphere strongly influenced by the agro-ecosystem conditions such as the type and altitude of cover crops and cultivation techniques. Under these conditions, an attempt to find entomopathogenic fungi that can play a role in the control of pod borer peanuts are easy and appropriate for farmers to be very important. This research aims to study and know the pod borer attack, and to study the diversity of entomopathogenic fungi around the roots which are peanuts that have the potential to control pod borer larvae in West Sumatra.

II. MATERIALS AND METHODS

A. Survey of Peanut and Incidence of Pod Borer Attack

Study of pod borer attack was conducted from April to August 2012. Survey was conducted by purposive sampling method in three subdistricts in Pasaman Barat, West Sumatra Province. In every district observed two planting area that

almost harvested. Each of which was observed as many as 80 plant samples. All plant samples were collected then taken to the laboratory for counting pod damage, the symptoms of the attack, as well as observing the pod borer larvae.

B. Collection and Isolation of Entomopathogenic Fungi

Study of entomopathogenic fungi was conducted from November to December 2012 in the peanut-producing several districts in West Sumatra, the West Pasaman, Tanah Datar, Solok, Agam and the South Coast. Description of sites has shown in Table 1. The method used to collect soil entomopathogenic fungi of groundnut crop is stratified purposive sampling method. Collection is done on five district centers groundnut production in West Sumatra. In each district selected one location and at each of two locations were selected for sampling farmers' fields. Bean crop land selected is land previously planted peanuts.

Before entomopathogenic fungi isolated from soil planting peanuts, which are first ground around the roots of plants collected by excavated soil at a depth of 10-15 cm in the soil around the roots of bean plants using a small shovel. At each sampling site were taken 5 sample points specified diagonally. At 5 points are taken soil samples of 500 grams. Soil samples were then inserted into a plastic bag and labeled and stored in the cooler box. Soil samples were then taken to the laboratory for further processing. Isolation of fungi from soil was conducted using traps (bait method) using *Tribolium molitor* larvae and use the dilution method.

To isolate the fungus from the soil by trapping method (verse method), soil samples from each site cleared of plant roots, then crushed and sieved with a 600 mesh sieve. Land that has been treated earlier as much as 500 grams inserted into a plastic box measuring 15 cm x 10 cm x 10 cm. Soil moistened with sterile water until the soil looks a little wet, then put 10-instar larvae of *T. molitor* 5 newly molted. The box is then incubated at room temperature (25°C) for 10 days. Observation of *T. molitor* larvae infected performed every day. Dead larvae were removed and surface sterilized with 1% sodium hypochlorite and rinsed 3 times with sterile water. Further input into the petri dish was covered with moist filter paper and incubated for 12 days. Isolation of fungi from infected larvae done by taking conidia of fungi that grow on the outside of the body and the larvae were grown on medium SDAY and then purified.

Isolation of entomopathogenic fungi from soil was also carried out by the method of dilution by taking from each soil sample as much as 10 grams, then dissolved in 100 ml of sterile akuadest who has given 0.05% tween 80 and in the vortex for 2 minutes. Soil suspension diluted (serial dilution) up to 3 times and 0.1 ml of the suspension included in a petri dish containing medium has SDAY for isolation of entomopathogenic fungi. Petri dishes were incubated for 5 days and colonies of fungi isolated were there back then purified on SDAY media. Identification of fungi obtained macroscopic and microscopic done. Identification key is a key used Barnett and Hunter (1972), Poinar and Thomas (1984), and Watanabe (2002).

TABEL 1
SITES OF SAMPLE COLLECTION OF PEANUTS RHIZOSPHERE SOIL
IN WEST SUMATRA PROVINCE

Sites	Cropping system	Species of Plants
Nagari Lolo, district of Pantai Cermin, District of Solok	polyculture	peanut, banana, corn, manihot
Nagari Muaro Kiawai, subdistrict of Gunung Tuleh, district of Pasaman Barat	polyculture	peanut, sugarcane, citrus, corn, banana
Nagari Sungai Buluh, sub district Matur, District of Agam	Monoculture, and polyculture	Peanut, corn
Nagari Balimbiang, sub district Rambatan, district of Tanah Datar	monoculture, and polyculture	Peanut, corn
Nagari Ganting Mudiak sub district of Sutura, district of Pesisir Selatan	monoculture	peanut

III. RESULT AND DISCUSSION

A. Description of *Etiella zinckenella* and Its Attacking on Peanut

The larval form of *E. zinckenella* found is cylindrical shaped body and segmented, each segment there is a pair of legs, body length 3- 12 mm consisting of several instars, larval color green to red and black on the head. This body of larval instars can be seen in three to five instars.

In each pod attacked found hole size 1-1.5 mm. Holes generally at the base, middle and end of the pod. When pods peeled, seeds obtained were damaged partially or completely mixed with feces (Fig. 1). The larvae can devour whole seed, but usually only partially. Meanwhile, the remaining seed live saprophyte fungi that enter through the hole. Moreover, inside each pod always found feces larval support for the growth of saprophytic fungi. The presence of this fungi degrade the quality of seed so it can not be harvested at all.

Early instar usually entered by punching holes in the base ginofo (Apriyanto, 2010). Nearly all of the live larvae are inside the pod. Nevertheless, the possibility of the larvae can move from one pod to other pods. Late instar larvae more active so that it can move and destroy more than one pod in this phase. According to Djuwarso and Hartono (1998), *E. zinckenella* in each pod found only one larva because if more than one they will compete and cause one of them will move to the other pods. With such behavior tends to cause high levels of pod damage. Larvae were found to generally measure 8-10 mm with varying colors of green and reddish. Based on the size, the larvae belong to the 4th and 5th instar



Fig 1. Holes on peanut pod and larvae feeding inside in pod (above), faeces remaining inside pod, and the 4th instar larvae

B. Incidence of Pod Borer Attack in West Pasaman

Survey was conducted from April to August 2012 showed differences in the level of pod borer attacks on peanut plants in several locations (Table 2). All of clumps (100%) was attacked in Sungai Aua and Koto Balingka. Pod attacked was higher At the these locations grown improved varieties (Domba), but at Gunung Tuleh that grown Domba and local variety not all attacked. Local varieties have tissue harder and more dense than Domba. Local varieties have a potential genetic resistance should be developed. Low rainfall during April to August 2012 to support the development of pod borer population. It was also reported that population higher in dry season than wet season on soybean in West Java and Midle Java (Soekarna and Tengkan, 1979).

TABLE II
THE PERCENTAGE OF CLUMPS ATTACKED, PODS ATTACKED AND DAMAGED SEEDS IN THREE SUBDISTRICTS IN WEST PASAMAN

Sub Districts	Clumps attacked (%)	Pods attacked (%)	Damage seeds (%)
Gunung Tuleh	70	14.4 – 26.1	15.9
Sungai Aua	100	61.1- 64.4	27.3
Koto Balingka	100	67.6 – 72.6	51.1

C. Fungi of Groundnut's Rhizosphere as Potentially Entomopathogenic

Entomopathogenic fungi infection rates in *T. molitor* larvae depend on ground source or location (Fig.2). Of the four areas sampled, the percentage of *T. molitor* larvae

infected with entomopathogenic fungi ranged between 8-30%.

In addition to using the method of trapping insects with larvae of *T. molitor*, the isolation of entomopathogenic fungi from rhizosphere peanuts also performed with dilution method. Based on the results of the collection and identification of the isolates showed that the three regions found five sampling locations genus of entomopathogenic fungi that can be isolated from soil, namely *Metarhizium* (Deuteromycotina: Hyphomycetes), *Trichoderma* (Hypocreales: Hypocreaceae), *Aspergillus* (Eurotiales: Eurotiaceae), *Fusarium* (Hypocreales: Nectriaceae), and *Paecilomyces* (Eurotiales: Trichocomaceae). Based on the research results that have been obtained show that there are two types of the most dominant entomopathogenic fungi found in the peanut crop ecosystems *Aspergillus* and *Metarhizium* group. Both types of entomopathogenic fungi is found in all locations of planting peanuts. The genus *Paecilomyces* was found only in the location of the peanut crop in the propagation area. Some isolates were successfully collected can be seen in Table 3.

TABLE III
RESULTS OF A COLLECTION OF ENTOMOPATHOGENIC FUNGI FROM THE RHIZOSPHERE OF PEANUT AT VARIOUS LOCATIONS

Regions of Origin	Genus	Number of isolates
Matur (District of Agam)	<i>Trichoderma</i>	2
	<i>Aspergillus</i>	2
	<i>Metarhizium</i>	4
	<i>Fusarium</i>	1
Rambatan (District of Tanah Datar)	<i>Trichoderma</i>	2
	<i>Aspergillus</i>	8
	<i>Metarhizium</i>	14
	<i>Fusarium</i>	3
	<i>Paecilomyces</i>	2
Gunung Tuleh (District of Pasaman Barat)	<i>Aspergillus</i>	13
	<i>Metarhizium</i>	2
	<i>Fusarium</i>	4

The percentage of *T. molitor* larvae exhibiting symptoms of mycosis mostly in the peanut crop land from areas Surian (Solok district), which reached 30%, and the lowest are found in the soil planting peanuts originating from the propagation (Tanah Datar) i.e 8%. One of the causes of the high rate of infection in the entomopathogenic fungus *T. molitor* larvae of this is the high number of infective propagules in the soil. Tanada and Kaya (1993) suggested that the size of the inoculum density of the pathogen or is one of the important factors for the occurrence of infections in insects. In addition, factors may also affect the use of pesticides, to infection by entomopathogenic fungi. Intensive use of pesticides in areas negatively affect the propagation of entomopathogenic fungi against insect infections. The results of the study Sapieha-Waszkiewicz *et al.* (2005) found that the intensity of entomopathogenic fungi infection in *Galleria mellonella* larvae was higher in the soil planting grapes that are not applied with pesticides (72.5%) compared with the planting soil with pesticides applied wines (32.5%). In addition to pesticides, soil tillage intensity also affects the population of entomopathogenic fungi in soil. The results of

the study Sosa-Gomez *et al.* (2001) showed that the population of entomopathogenic fungi in soil soybean higher in untreated soil compared to the cultivated land.

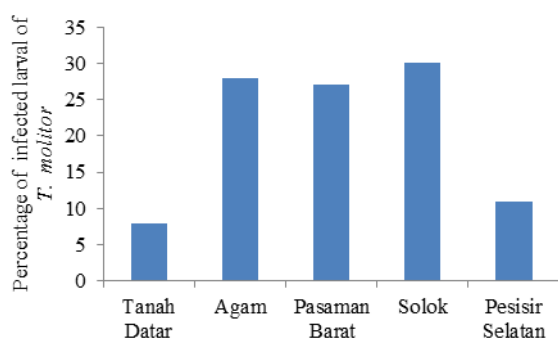


Fig.2. Mortality of *T. molitor* larvae infected with entomopathogenic fungi from several of soil collection sites

From the results obtained is also shown that in addition to entomopathogenic fungi *Metarhizium* and *Aspergillus*, other fungi found in soil throughout the sample site is *Fusarium*, while other entomopathogenic fungi such as *Beauveria bassiana*, *Verticillium* which can usually be found in the rhizosphere of plants, not found in the location sampling. Barker and Barker (1998) suggested that entomopathogenic fungi *B. bassiana* and *M. anisopliae* is more often found in grassland soil compared to forest land and agricultural land, while *Paecilomyces cf. cicadae* only found in forest soils. This shows that *Metarhizium*, *Aspergillus* and *Fusarium* fungus is commonly found in soil and agricultural land where the fungus is also influenced by the location of the area. Samer research results (2011) get 4 types of rhizosphere chili entomopathogenic fungi, namely *Fusarium*, *Aspergillus*, *Trichoderma* and *Metarhizium*. In the area of flat ground and Solok found 3 types of fungi, while in Padang only 2 types of fungi were found. *Fusarium* and *Aspergillus* are found in all rhizosphere chili crop, whereas *Trichoderma* was found only in one location in Tanah Datar and *Metarhizium* in Solok. The results of the study Lezama-Gutierrez *et al.* (2001) showed that the ground maize and sorghum in the three Mexican states found that *M. anisopliae*, *Beauveria bassiana* and *P. fumosoroseus*. *M. anisopliae* was the most dominant species, found 25% of the entire sample locations, whereas *P. fumosoroseus* was only found in one location sampled (5%). Bidochka *et al.* (1998) also reported that there are three types of entomopathogenic fungi found in soil samples from various locations in Notario (Canada) that *M. anisopliae*, *B. bassiana* and *Paecilomyces*. *M. anisopliae* is more commonly found on agricultural land while *B. bassiana* more commonly found in natural soil (natural). Molina-Ochoa *et al.* (2003) further found that from ground maize in Mexico found two types of entomopathogenic fungi *B. bassiana* and the *M. anisopliae*. Entomopathogenic fungus *M. anisopliae* is a type of fungus which is predominantly found in almost all locations.

The propagation from district of Tanah Datar has a species diversity and abundance of entomopathogenic fungi higher than in other areas, namely the five genera, while species diversity of entomopathogenic fungi found in the

area of West Pasaman, the three genera. At least the type and number of fungi found in soil allegedly influenced by the physical and chemical properties of soil, altitude, soil temperature, crop types and cultivation techniques. In the area of the District. West Pasaman intensity of land management, the use of chemical fertilizers and pesticides are more intensive land for palm oil so it can also affect crop land planted peanuts, so it can affect the existence and distribution of entomopathogenic fungi in the soil. Existence, diversity and distribution of entomopathogenic fungi in soil is strongly influenced by soil organic matter content, pH and soil type, altitude, habitat, soil temperature, geographic location, environmental conditions, type of crop and cultivation practices (Ali-Shtayeh *et al.*, 2003 ; Barker and Barker, 1998; Lezama-Gutierrez *et al.*, 2001; Molina-Ochoa *et al.*, 2003). The use of pesticides in the cultivation of peanuts can also suspected to influence the diversity of entomopathogenic fungi. Intensive pesticide application on groundnut crop can affect the survival of conidia in soil. According Sapieha-Waszkiewicz *et al.* (2005) the presence of entomopathogenic fungi in soil is highly dependent on cultivation techniques and the use of pesticides Sudarmadji (1994) suggested that entomopathogenic fungi *B. bassiana* is very sensitive to the fungicide and insecticide BPMC mankozeb and somewhat sensitive to the insecticide endosulfan.

IV. CONCLUSIONS

Damaged by pod borer in peanuts has a characteristic holes ranging in diameter from 1 to 1.5 mm located either at the base, middle and end of the pod irregularly. The larvae can devour whole seed, but usually only partially and found saprophyte fungi in the remaining seed. Incidence of pod borer attacking on groundnut was 14 to 70%. *Metarhizium sp.*, *Aspergillus sp.*, *Trichoderma*, *Fusarium* and *Paecilomyces* were groups of fungi as entomopathogenic successfully isolated from soil of groundnut's rhizosphere and they were able to infected of *Tribolium molitor* 8-30%.

ACKNOWLEDGMENT

We thank to the Higher Education of General Directorate funded by Competitive Fund Research 2013-2014

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